

Characterization of white grub (Melolonthidae; Coleoptera) in salak plantation based on morphology and protein banding pattern

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Abstract. Maryati KT, Sugiyarto. 2010. Characterization of white grub (Melolonthidae; Coleoptera) in salak plantation based on morphology and protein banding pattern. *Nusantara Bioscience* 1: 72-77. This research aims to find out the white grub (Melolonthidae; Coleoptera) variability based on the morphological characteristic and protein banding pattern found in "salak pondoh" farm in Regencies of Sleman, Yogyakarta and Magelang, Central Java. Each area has five sampling points. Morphological analysis on white grub was conducted using descriptive method and analysis on protein banding pattern was conducted using qualitative analysis based on the presence or absent of band pattern on the gel, and qualitatively based on the relative mobility value (Rf) of protein. The result indicated that the white grub in Sleman and Magelang, based on morphology characteristic is only one species, namely *Holotrichia* sp. Based on the protein banding pattern, the white grub sample have differences of protein band number and protein molecular weight.

Key words: *Salacca zalacca*, white grub, morphology, protein banding pattern.

Abstrak. Maryati KT, Sugiyarto. 2010. Karakterisasi lundil putih (Melolonthidae: Coleoptera) pada pertanaman salak berdasarkan ciri morfologi dan pola pita protein. *Nusantara Bioscience* 1: 72-77. Penelitian ini bertujuan untuk mengetahui keanekaragaman lundil putih (Melolonthidae; Coleoptera) berdasarkan ciri morfologi dan pola pita protein yang ditemukan di lahan pertanaman salak pondoh di Kabupaten Sleman, Yogyakarta dan Kabupaten Magelang, Jawa Tengah. Pada masing-masing wilayah diambil lima titik sampling. Analisis morfologi lundil putih digunakan metode deskriptif, dan analisis pola pita protein digunakan analisis kualitatif berdasarkan muncul tidaknya pola pita pada gel, dan secara kuantitatif berdasarkan nilai mobilitas relatif protein (RF). Hasil penelitian menunjukkan bahwa sampel lundil putih di Kabupaten Sleman dan Magelang, berdasar karakter morfologi hanya satu spesies yaitu *Holotrichia* sp. Karakter pola pita protein sampel lundil putih dari Sleman dan Magelang mempunyai perbedaan jumlah pita protein dan berat molekulnya.

Kata kunci: salak, lundil putih, morfologi, pola pita protein.

INTRODUCTION

Salak pondoh from around Mount Merapi (Sleman District, Yogyakarta and Magelang Districts, Central Java) is a kind of superior fruit in Indonesia, which requires the support of research in developing a system of cultivation. One problem that needs attention is the frequent appearance of pest. Pests are all biotic organisms or agents that damage crops or crop yields in ways that conflict with human interests (Oka 1995). One of the groups that are very harmful pests salak pondoh farmer have had and have not found a way of intensive control is a white grub (Java: uret/embog). White grub Scarabidae members in particular and generally behave as a plant pest (phytophage) as well as a decomposer (saprophage). According Sugiyarto (2008), white grub on salak pondoh cultivation area on the slopes of Mount Merapi is *Holotrichia javana* Brsk. Wardani and Sugiyarto (2009) found genetic variation on white grubs population at salak pondoh agroecosystem in slope of Mount Merapi based on esterase and peroxidase isozymes.

In the phase of white grub larvae eat various kinds and forms of organic substances (multi-phytophage). In nutrient rich habitats or organic materials, white larva tend to eat rotting wood, dung of other animals and other organic materials. In the habitat with limited organic matter content, white larva tend to eat roots and stem tubers of plants or plants that exist (Borror et al. 1992; Chu and Cutkomp 1992). Pracaya (1999) suggested that at the outset of this pest it only eats humus and other debris, but after a little big and it then eats the roots of plants that are still alive, sometimes even eating tree bark in the soil so that it can cause plant death.

Until now there has been known no complete information about the character type of white grub pests. So far its control efforts have been made through various approaches such as physical and chemical approaches, but the results are not satisfactory. In order to develop a biological control (biology), the key to success is the presence of complete information on the characteristics of the specimen. Until now there has been known to complete information about the characteristics of white grub, which

specifically attacks the bark of plants in Sleman and Magelang. Therefore, it is necessary to know characterization of these pests.

With the development of molecular biology studies, the introduction of the specimen leads to intracellular components as the clues. One of them is the analysis of proteins by electrophoresis analysis method. Charged proteins will undergo a process of separation in an electric field. Electrophoresis is an extremely important method in the process of separating the molecules, especially proteins. Because, besides this method has no effect on its biopolymer structure, is also very sensitive to the charge and molecular weight differences which are quite small (Bachrudin 1999).

With the techniques of electrophoresis, the protein molecules can be separated into several parts according to its molecule weight that form bands that can be used to determine the variation in a population and the degree to accuracy is very high and very suitable to test the diversity at the population level (Setyawan et al. 2002). Therefore the introduction of morphological characteristics and protein banding pattern on the white grub is needed in order to determine appropriate pest control techniques. This study aims to determine the variety of white grub (Melolonthidae; Coleoptera) based on morphological characteristics and protein banding pattern found in crop salak pondoh in Sleman, Yogyakarta and Magelang regency, Central Java.

MATERIALS AND METHODS

Time and place

The experiment was conducted from August-November 2007, consisted of two activities, namely: white grub larvae sampling conducted in Bangunkerto Village, Turi Subdistrict, Sleman District, Yogyakarta and Dadapan Village, Srumbung Subdistrict, Magelang District, Central Java. Meanwhile, the laboratory studies conducted at the Sub-Lab Biology, Central Laboratory of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta which aims to determine the morphological characteristics and protein banding pattern of white grub.

Material

The material used is the white grub from Sleman, Yogyakarta, and Magelang District, Central Java. SDS-PAGE with Comassie blue dye used to reveal the diversity of protein bands.

Procedures

Sampling of live specimens of white grub in salak pondoh plantation area in two areas namely Sleman and Magelang conducted with purposive random sampling method, where each region with a five-point sampling. Sampling was done by placing a square of size $1 \times 1 \text{ m}^2$ at each sampling point, i.e. in the vicinity of salak pondoh suspected white grub pest attacks, then made excavation with $\pm 30 \text{ cm}$ depths.

White larva taken from the soil by hand sorting method.

The white larvae were collected in the form of live specimens in a way which maintained the soil medium and it was placed in a container (medium soil taken from soil excavation). Selected relatively uniform in size about 3 cm, a minimum of 10 specimens from each location, to observe the morphological characteristics and protein banding pattern was analyzed.

Environmental parameters. Besides collecting the white grub, at each sampling point was also made observations of habitat characteristics that include

Air temperature. Measurement of air temperature was by using a thermometer placed one meter above ground level for ± 5 minutes.

Soil temperature. Measurement of soil temperature was by using a thermometer that is attached to the soil as deep as 10 cm soil depth for ± 5 minutes.

Soil acidity (pH). Acidity (pH) were measured by using pH-meter gauge or soil tester is plugged directly into the soil $\pm 10 \text{ cm}$ for ± 5 minutes.

Soil moisture content. Soil water content was calculated by means of twenty grams of soil (wet weight) is inserted into the oven at 105°C for two hours, then removed and weighed in the light dry weight. Wet weight minus dry weight divided by wet weight and multiplied by one hundred percent represents the percentage of soil moisture content (Suin 1997).

Soil organic matter. Measurement of soil organic matter carried by Walkey and Black method, namely: Samples of 1 g of dry soil put into 50 mL of cook glass jar. Then add to it 10 mL of concentrated H_2SO_4 and, $\text{K}_2\text{Cr}_2\text{O}_7$ -IN. Settling for 30 minutes. Then add 5 mL of concentrated H_3PO_4 and diluted with akuadest until a pumpkin drinks and rocked back and forth, and then precipitated. The part that clearly taken as many as 5 mL and put into 50ml erlemeyer, then added 15 mL akuadest. Added 2 drops of indicator DPA. Then in the titration with FeSO_4 0.5 N until it changes color (greenish-blue). As a comparison made also the blank solution.

$$\text{Organic C} = \frac{(b - a) \times N \text{ FeSO}_4 \times 3 \times 10 \times 100/7}{(100/100 + k_a) \times \text{sample (mg)}}$$

$$\text{Organic matter C} = \text{organic C} \times 100/58$$

a = standard solution (with soil),

b = volume of blank solution (without soil),

k_a = water content (Afandie 1987).

Observation of morphology of the white grub is assisted by a magnifying glass, to identify the morphological characteristics of hair distribution, the number of segments, legs, labrum shape, the shape of maxilla, shape nails and body size. (Chu 1992; Penebar Swadaya 2005). Then the morphological data was documented through photography and was tabulated by diversity of analysis. (Wiratno et al. 1997).

Protein analysis was conducted using SDS-PAGE (Suranto 2003) with Comassie blue dye.

Extraction of samples. White grub, through surgery, was removed its digestive tract, weighed, and then

destroyed by mortar porcelain, added the buffer before the extract with a ratio of 1: 3. Following extract included in the Eppendorf to centrifuged at 10,000 rpm for 5 minutes and 30 seconds. Sample solution were separated into two parts, the top node (supernatant) to be used in the process of electrophoresis and stored at a temperature of -40°C, while the bottom in the form of solid material (pellets) are removed.

Preparation of extract buffer, as follows: given a buffer tank of acid as much as 0.288 g borax and 0.63 g of borax, distilled water added up to 40 mL. Extraction buffer consisting of 10 g sucrose, 0.42 g ascorbic acid, 0.036 g cysteine and 40 mL buffer tank mixed together, pH. 8.4.

Preparation of gel solution. Solution of "L" in the form of 9.066 g Tris and 0.2 g of SDS dissolved in 40 aquabidest (twice distilled water), above the pH to 8.8, with the added HCl and aquabidest order volume 50 mL. Solution of "N" of 175.2 g of acrylamide, and 4.8 g bis-acrylamide was dissolved in 40 mL aquabidest until the volume reached 60 mL. Solution of "M" of 1.51 g Tris and 0.1 g SDS was dissolved in 20 mL of distilled water, pH set to 6.8 to 7.0 with HCl and aquabidest added to 25 mL volume. Dye loading of 250 mL glycerol and 50 mL bromphenol blue was dissolved with 200 mL of distilled water, dye loading function to include samples in the wells as ballast (Suranto 2003). Furthermore, the manufacture of gel, using the following conditions: 1.235 mL of "L" solution, plus 2.054 mL of "N" solution, 1.625 mL of distilled water, 5 mL Temet, and 15 uL Aps mixed into one (Suranto 2002).

Prepare the gel mold. Assembled gel mold is equipped with glass mold space/divider in place behind the smaller glass mold. Mold casting glass was mounted on the frame and then was put on the casting stand.

Gel formation. Solution to gel was poured in molds and then added the saturated isobutanol. The gel is formed approximately 45 minutes. Saturated isobutanol disposed absorbed with blotting paper. Gel that had formed was transferred from the cleaning frame to the electrophoresis tank.

Electrophoresis. Electrophoresis was conducted in tank content with running buffer until full. And then the supernathan of larvae samples were taken with a micropipette as much as 7 mL. 3 mL of it, with loading dye was mixed in paraffin paper, included in the gel wells carefully, after all samples in the wells, plug cap and set the voltage electrophoresis (100 v, 90 minutes) and then executed. Running buffer used: TAE 10 X diluted to 1 X TAE with distilled water. A 1-time running was required 1000 mL.

Staining proteins. Comassie blue solution was made with the composition of 1 g Comassie Blue dye was dissolved in 100 mL of acetic acid plus 400 mL of methanol and then diluted with the addition of distilled water until it reached the volume of 1 L. While the distaining solution was made with a composition of 100 mL of acetic acid plus 400 l of methanol and diluted with the addition of distilled water until it reached the volume of 1 L (Rickwood and Hames 1990). Gel that has been released from the mold and has underwent running, then was soaked in dye solution *Comassie* Blue for 12 hours, then washed with distaining as many as 3-4 times for 2

hours to obtain the protein banding pattern that was formed then the gel could be recorded in picture/tape to digital photos.

Making zimogram. The patterns of protein bands have been recorded and then tape the pattern drawn on graph paper, measuring the migration distance (Rf) were measured from a distance tape apparently divided loading dye migration distance.

Data analysis

Analysis of the data used in this study was using cluster analysis. According to Santoso (2004), cluster analysis aims to classify the objects based on common characteristics. The object will be classified into one or more cluster (group) so that objects that are in one cluster would have a resemblance to one another. Cluster analysis is very suitable applied in the field of biology, especially in helping the process of taxonomy to classify a particular organism. Data analysis was applied in this study as follows:

The morphology of the white larva analyzed with descriptive methods. Identification of morphological characteristics included leg segments, labrum, maxilla, nail, body size, and distribution of fur (Chu and Cutkomp 1992), and then documented through photography, plainly morphological data in tabulation and diversity analysis (Wiratno et al. 1997).

The observation of protein band pattern that was qualitatively analyzed was based on whether the tape appeared on the gel. The diversity of banding pattern is determined based on the value of Rf (Riesenberg et al. 1987; Suranto 2002). Value of relative protein mobility (Rf) was determined by the equation below (Rothe 1994).

$$Rf = \frac{\text{Protein migration distance}}{\text{Loading dye migration distance}}$$

Subsequently it was converted into binary data with a given value of 0 for the band that did not appear and was given the value 1 for a tape that appeared. They made a dendogram kinship with hierarchical cluster analysis using average linkage method (between group) SPSS (Santosa 2003)

RESULTS AND DISCUSSION

Environmental factors

Environmental factors in Sleman and Magelang, including: altitude, air temperature, soil temperature, soil pH, soil moisture, and soil organic matter factors are shown in Table 1. The temperature at the sampling point Sleman and Magelang have the same environmental parameters. This is evident from the calculation of the independent T test that has a t value 0.929 with probability 0.380; where the probability value above 0.05. Thus the air temperature in Sleman and Magelang has no significant difference. Likewise, soil temperature and soil pH, all have the same environmental parameters. This is evident from calculating the probability value above 0.05. But the soil moisture content have significant differences. This is evident from the value t calculate equal -5773 with a probability of 0.000

where the probability value is below 0.05. So the soil water content between Magelang and Sleman is different, where a higher water content is in Magelang. Magelang has an average of 19239.000 while Sleman has an average 12770.000. This can be understood because in Magelang there are many plantation weeds and shrubs to reduce evaporation. Other crops are not a lot so that transpiration is also small. In Sleman there are no weeds and bushes so that transpiration and evaporation is big.

Based on the height, Magelang has a height of 545 m above sea level, Sleman has a height of 484 m above sea level. Based on soil organic matter, Sleman have higher soil organic matter which is 5.52 while soil organic matter in Magelang is 3.605. Soil organic matter (BOT) is the remains of plants and animals in the soil that broken down into humus. Sleman has higher soil organic matter in comparison with Magelang. Because Sleman has more of vegetation of salak pondoh that is made for ecotourism, where the number and types of plants has to be looking good. While Magelang, salak garden is maintained by population according to the ability of the population.

Table 1. Environmental parameters in the field sampling of white grub in Sleman and Magelang

Environmental variables	Sleman	Magelang
Altitude	484 m dpl	545 m dpl
Organic matter (%)	5.52	3.605
Air temperature ($^{\circ}\text{C}$)	30.88	29.68
Soil temperature ($^{\circ}\text{C}$)	27.3	25.8
Soil pH	6.72	5.44
Soil water content (%)	12.770	19.239

Morphological characteristics

The observation of white grub morphological features of two locations: the location I Turi Sleman, Yogyakarta-II and locations of Magelang, Central Java, are described in Table 2 and Figure 1.

Table 2. Results of morphological identification of white grub from Sleman and Magelang

Character	Sleman	Magelang
Body color	White	White
Thoracic vertebra	3 segment	3 segment
Abdominal segment	10 segment	10 pairs
Number of legs	3 pairs	3 pasang
Number of leg segments	4 segment	4 segment
Upper lip	No segment	No segment
Maxillary	Galea-Lacinia united	Galea-Lacinia united
Nails tibiotarsus	Split in two	Split in two
Body size	2,6-2,9 cm	2,5-3,0 cm
Feathers distribution	Evenly	Evenly

Environmental parameters in Sleman and Magelang, based on the calculation of unpaired t tests showed that air temperature, soil temperature and soil pH have significant differences, but higher soil moisture content in Magelang and higher soil organic matter in Sleman. With these

environmental conditions, there was no difference in the sample of white grub in Sleman and Magelang, based on morphological characters.

From the larvae observation in Sleman, Yogyakarta and Magelang, Central Java, under the 3rd character on foot segment comprises four segments, labrum (upper lip) merge into a single/not segmented, Galea and lacinia in the maxilla merge into one, that is characteristic of the family Scarabaeidae (Chu and Cutkomp 1992). Based on the character of the nail on tibiotarsus jagged or torn in two (bifid) is one of the sub family Melolonthinae (Borror et al. 1992). While based on body size between 2.5 to 3.0 cm is characteristic of Holotrichia sp. Based on the characters mentioned above, samples of white grub at two locations namely Sleman, Yogyakarta and Magelang, Central Java is the same.

Phenotypes in living organisms is a combination of factors genotype and environmental factors (Prawoto et al. 1987). Although Sleman and Magelang are two different environments, but the phenotypes that emerged in the form of morphological characters in Sleman and Magelang larva samples are the same. This is due to the phenotypes that arise not necessarily be a character of morphology, physiology can also be a character. The change in character only affects the physiology of the cell performance system (Brooker 1999). So it can not be detected at the level of morphology.

Another possibility is the absence of differences in morphological characteristics between Sleman and Magelang though the environment is different, due to genetic factors have a stronger influence than environmental factors. According Suranto (2001) the emergence of variation can be caused by two factors: environmental and genetic factors. If genetic factors have a stronger influence than environmental factors, then the creatures living in an environment though different show no morphological variation.

Character protein band

White grub from Sleman

Proteins can be separated by using electrophoresis. The results of polyacrylamide gel electrophoresis of protein samples from Sleman larva, are shown in Figure 2. Zimogram of protein banding pattern (Figure 2) express 5 (five) bands at Rf 0.016, 0.067, 0.101, 0.135, 0.372. Band numbers 1, 2, 3, 4, 5, found in all samples larva of Sleman. The difference lies in the Rf value of 0.101 in larva samples 3, 4, 7, 8, 9, 10 expressed thicker than bands.

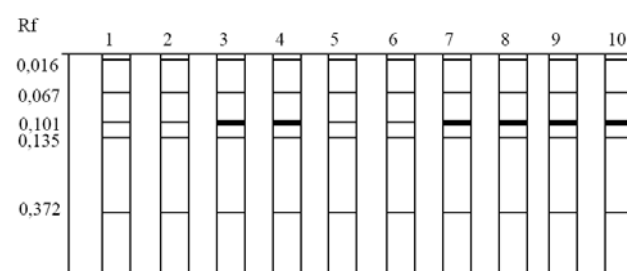


Figure 2. Zimogram protein in samples of white grub from Sleman.

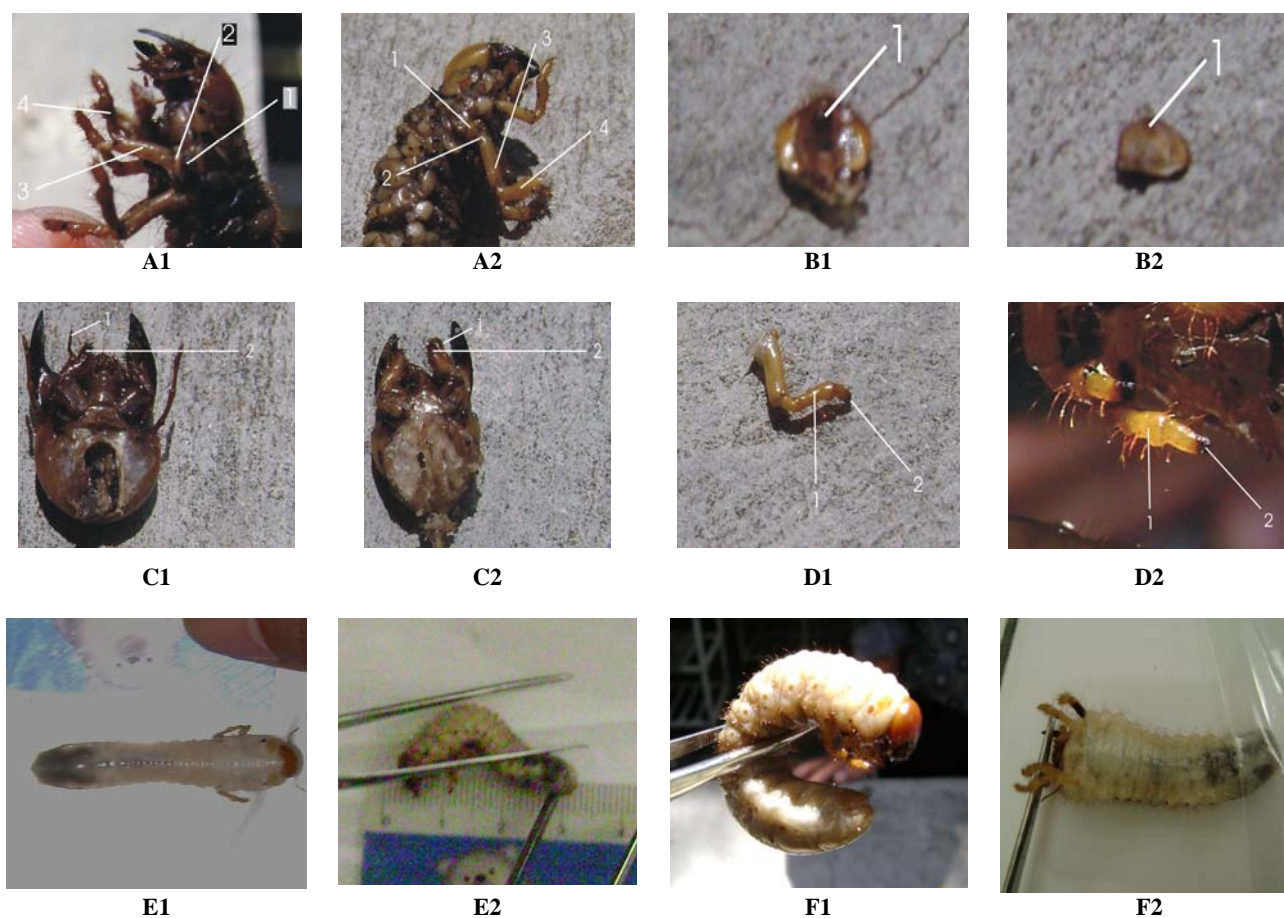


Figure 1. A. Legs white grub, consisting of 4 segments. 1. Coxa, 2. Trochanter, 3. Femur, 4. Tibiotarsus. B. Labrum (upper lip) blend of white grub (not segmented); 1. Labrum (upper lip) are not segmented. C. Galea and Lacinia of maxilla fused white grub; 1. Palp, 2. Galea and Lacinia. The three morphological characters above are some characteristics of the family Scarabaeidae (Chu and Cutkomp, 1992). D. Nails on white grub tibiotarsus bergeligi or split in two (bifid); 1. Tibiotarsus, 2. Nails bifid. This is characteristic of sub families Melolonthinae (Borror et al. 1992). E. White larva body length from 2.6 to 2.9 cm (body length *Holotrichia* sp. Maximum 3 cm). F. Spread evenly coat of white grub, which was identified as *Holotrichia* sp. Description: Image of the left is a sample of Sleman (1), the right is a sample of Magelang (2).

Based on the difference in whether or not the tape appeared thin and thick bands, the white larva of Sleman samples tested found that there are 2 (two) banding pattern (Figure 2) namely: Pattern band A larva is owned by the sample numbers 1, 2, 5, 6. That is, the sample larva 1, 2, 5, 6 has a protein band at Rf value of 0.016, 0.067, 0.101, 0.135, 0.371 with the same thickness of tape and band pattern B being owned by the larva samples number 3, 4, 7, 8, 9, 10. This means that larva samples 3, 4, 7, 8, 9, 10 have bands at Rf 0.016, 0.067, 0.101, 0.135, 0.371 with a thickness that is not the same band with at Rf 0.101. The thickness of the different bands do not indicate a different molecular weight protein but only the amount of protein with differently migrated. Larva samples tested so that there is no variation based on protein banding pattern. Results of protein analysis showed that the protein band profiles of samples tested from Sleman larva showed no genetic variation, seen in the bands that are expressed in same time, both in quantity and its Rf value. While the difference in thickness of thin bands due to differences in the number of protein molecules that migrated or

differences in the content/quantity of protein (Supriyadi et al. 2004; Supriyadi 2006).

White grub from Magelang

The results of polyacrylamide gel electrophoresis of protein samples from Magelang larva, shown in Figure 3. Zimogram protein bands in (Figure 3), expressing six bands at Rf 0.016, 0.047, 0.079, 0.190, 0.317, 0.372. The six present in all samples larva from Magelang. Difference bands at Rf 0.047 and 0.079 Rf was expressed thick in larva samples 5, 6, 7, 8.

Based on the nature of the differences appear whether or not tape (qualitative) and thickness of thin band (quantitative), white larva samples from Magelang location there are 2 patterns of protein bands (Figure 3). Banding pattern A and pattern B bands respectively express 6 bands with the same Rf value. A banding pattern is owned by the larva samples 5, 6, 7, 8. The sample grub 5, 6, 7, 8, has a protein band at Rf 0.016, 0.047, 0.079, 0.190, 0.317, .372, with bands of different thickness. The difference lies in the pattern of bands with Rf value of 0.047, 0.079. Banding

pattern of sample B is owned by the larva 1, 2, 3, 4, 9, 10. This means that larva samples 1, 2, 3, 4, 9, 10 had a protein band at Rf value of 0.016, 0.047, 0.079, 0.190, 0.317, 0.372; with the same thickness.

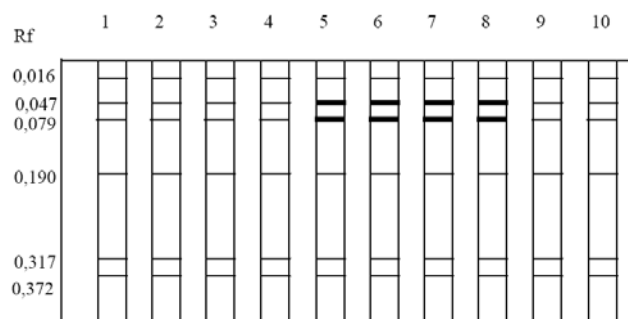


Figure 3. Zimogram proteins in samples of white grub from Magelang

Results of protein analysis showed that, the profile of protein bands from tested samples from Magelang larva showed no genetic variation, seen in the bands expressed in the same time, both in quantity and value of its RF. While the difference in thickness of thin bands was due to differences in the number of protein molecules that migrated or differences in the content/quantity of protein (Supriyadi et al. 2004; Supriyadi 2006), supported by a dendrogram that only one group, so the sample grub from Magelang based protein banding pattern only one, not diverse.

Protein banding pattern analysis results showed that each white grub populations from the same location shows no signs of genetic diversity, differing only in the thick-thin band, but if the banding pattern was comparable between the two populations then they are completely different. The interpretation of protein expression patterns of band samples of Sleman and Magelang grub is not the same. The big difference in protein expression that was on the number and value of Rf in both locations, shows the differences in genetic material between the samples from the larva of Sleman and Magelang. Although the morphological characteristics of the two locations together, i.e. one species, the possibility is the existence of morphological variation at the sub species. Another possibility of differences due to biochemical adaptation to environmental differences between Sleman and Magelang, so that the expression of proteins in two different locations.

CONCLUSION

White grub (Melolonthidae; Coleoptera) from Sleman regency, Yogyakarta and Magelang regency, Central Java, has the same morphological characteristics, and included in one species, namely *Holotrichia* sp. Based on the character of the protein banding pattern, the sample grub from the district of Sleman and Magelang district differ on the molecular weight of protein as well as the number of protein bands, so that genetic material is also different between the two possibilities.

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